The Nutritive Value of Dying Maize and *Setaria faberi* Roots for Western Corn Rootworm (Coleoptera: Chrysomelidae) Development

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ABSTRACT The timing that dying root tissues of *Setaria faberi* R.A.W. Herrm. and maize, *Zea mays* L., no longer support growth and development of neonate and second-instar western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), larvae was evaluated to enhance our understanding of the basic ecology of this pest. Three separate greenhouse experiments were conducted. In the first experiment, glyphosate was used to kill *S. faberi*. In the second experiment, glyphosate was used to kill maize, and in the final experiment, maize was killed by severing it below the growing point. These experiments evaluated western corn rootworm larvae for survival and growth parameters among living control plants, plants severed or sprayed on the day they were infested, plants severed or sprayed 5 and 10 d before they were infested, and plants planted 5 and 10 d early and severed or sprayed 5 and 10 d before they were infested (the last two treatments were controls for root size). Larvae were sampled from each of these treatments 5, 10, and 15 d after infestation, and beetle emergence was recorded from the remaining pots. When infested on the day of glyphosate spray, significantly fewer larvae were recovered from *S. faberi* than from living *S. faberi*. Overall, when infested 5 or 10 d after being sprayed with glyphosate or being severed below the growing point, no significant larval weight gain was recorded from any treatment. Host plant tissue apparently becomes unsuitable for larval growth within the first 5 d after glyphosate spray and severing below the growing point. The implications of these data toward current work involving alternate grassy hosts sprayed with herbicide, the increasing occurrence of volunteer corn, related studies on rootworm–host interactions, and certain adult emergence techniques are discussed along with possible mechanisms as to why the tissue becomes unsuitable so quickly.

KEY WORDS *Diabrotica virgifera virgifera*, nutritional ecology, plant–insect interactions

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), is a major pest in continuous maize, *Zea mays* L., fields in much of North America and Europe. It is primarily a pest in its larval stages, where it feeds on the roots of maize. Maize root damage can disrupt water relations (Riedell 1990), reduce nutrient uptake (Kahler et al. 1985), cause reductions in photosynthetic rate (Godfrey et al. 1993), and reduce plant stability (Spike and Tollefson 1989). In the adult stage, western corn rootworm beetles feed on maize silk and pollen, developing kernels, and a variety of other pollen sources that may be available (Moeser and Vidal 2005). Unlike the larval stage, western corn rootworm beetles do not usually cause significant yield loss, although under extremely high populations, pollination problems can result (Culy et al. 1992).

The nutritional ecology of western corn rootworm larvae is poorly understood for such a major pest. It is known that western corn rootworm larvae can develop on several grass species (Branson and Ortman 1967, 1970; Clark and Hibbard 2004; Oyediran et al. 2004; Wilson and Hibbard 2004). It is also known that plant phenology can affect development of western corn rootworm larvae on alternate hosts (Chege et al. 2005) and maize (Stavitsky and Davis 1997), where late-hatching larvae did poorly on early maturing maize. Why western corn rootworm larvae can survive and develop on some grass species, but not others is not known, nor is it known what aspects of older roots make them less suitable for western corn rootworm larval development.

In an elegant set of experiments, Moeser and Vidal (2004a,b) measured weight gain (or loss) of second instar western corn rootworm larvae and the amount of food consumed by the same individual larva to calculate food conversion indexes for a series of alternate hosts and maize varieties respectively. In addition, they evaluated the carbon/nitrogen ratio and the phytosterol content of the alternate hosts and maize varieties. For alternate hosts, the plant species with higher nitrogen content were less suitable for

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western corn rootworm development. Phytosterol content was positively correlated with the amount of food consumed, but not the weight gain of the insects. For maize, nitrogen content was positively correlated with converting root biomass into insect biomass, and phytosterol content influenced larval weight gain and the amount of infested food. The change in weight of the second-instar larvae evaluated after 6 d of feeding on severed roots ranged from strongly positive to strongly negative for different alternate hosts and different maize varieties. Presumably, a negative weight gain can result from not only a lack of feeding but also from a poor food conversion index. Differences were somewhat consistent among replications in both studies as indicated by the size of the error bars. Unfortunately, in both studies any differences in weight gain (or loss) of the larvae and the food conversion indices that were calculated are confounded with unknown differences in the speed that the alternate host species or maize variety decompose, because the same severed roots were the only food source for 6 d.

Dying maize plants have been evaluated in the field previously. One way to capture adults as they emerge from the soil is to sever the plant below the growing point (which kills maize plants) and to place an emergence cage over the location where the plant had been. Many types of emergence traps have been described previously (Tollefson 1986). Fisher (1984) evaluated adult emergence from the ground near maize plants that had been severed below the growing point and compared these data to adult emergence from living maize plants in the same field. He found that although severing the plants accelerated the time frame for adult emergence, the total number of adults emerged was not significantly different between severed and living plants when the timing of severing was initiated at a time when most larvae had pupated.

The timing that dying roots killed with herbicide become unsuitable for larval nutrition has recently become more important to consider. Oyediran et al. (2005) demonstrated that significantly more western corn rootworm adults emerged from transgenic, rootworm-resistant maize expressing the Cry3Bb1 protein when combined with grassy weeds (sprayed with glyphosate 4 d after infestation) than emerged from Cry3Bb1-expressing maize kept weed free or a living grassy weed control. Oyediran et al. (2005) assumed that neonate western corn rootworm larvae initially established on the grassy weeds and whenever the weeds became unsuitable due to the herbicide spray, the larger larvae were able to better survive the Cry3Bb1-expressing maize. The timing that alternate hosts, volunteer maize, or healthy maize severed for adult emergence studies become unsuitable for western corn rootworm larval growth and development is currently unknown. The goal of the current article was to determine the timing that dying root tissues of *Setaria faberi* R.A.W. Herrm. and maize become unsuitable for growth of neonate and second-instar western corn rootworm larvae.

Table 1. Treatment list for experiment 1[a,b]

Treatment no.	Treatment
1	Live *S. faberi* control
2	*S. faberi* sprayed with glyphosate on the day of infestation
3	*S. faberi* sprayed with glyphosate 5 d before infestation day
4	*S. faberi* sprayed with glyphosate 10 d before infestation day
5	*S. faberi* planted 5 d early and sprayed with glyphosate 5 d before infestation day[c]
6	*S. faberi* planted 10 d early and sprayed with glyphosate 10 d before infestation day
7	Live maize control

[a] Treatment list for experiment 2 was identical except that maize was used instead of *S. faberi* and there was not a seventh treatment.
[b] Treatment list for experiment 3 was identical to experiment 2, except that maize was killed by cutting it below the growing point instead of spraying it with glyphosate.
[c] Treatments 5 and 6 were intended as controls for treatments 3 and 4 for the effects of root size.

Materials and Methods

Western corn rootworm survival and growth parameters were evaluated among living control plants, plants killed on the day they were infested, plants killed 5 or 10 d before they were infested, and plants planted 5 or 10 d before all other plants and killed 5 or 10 d before they were infested (Table 1). The last pair of treatments served as controls for root size for the plants that were killed before infestation. There were three separate greenhouse experiments, which were conducted and analyzed separately. In experiment 1, glyphosate was used to kill *S. faberi* (Azlin Seed Service, Leland, MS). In experiment 2, glyphosate was used to kill maize (DKC 60–15, Monsanto Company, St. Louis, MO). In experiment 3, DKC 60-15 maize was killed by severing it below the growing point. Each experiment had two trials conducted during different months. Each trial had five replications of each treatment. The experimental design for each trial was a randomized complete block split-plot in space. The main plot was treatments (Table 1), and the subplot was sample times (larval recovery 5, 10, or 15 d after infestation). A fourth pot was used for adult emergence, but this was analyzed separately. In addition, each pot was infested with either newly emerged western corn rootworm larvae (<24 h old) or second-instar larvae, which doubled the size of the overall experiment.

Plant Growing Conditions. Seeds were planted in pots containing a 2:1 mixture of autoclaved soil/peat-based growing medium (Promix, Premier Horticulture LTEÉ, Quebec, ON, Canada). Clay 3.8-liter pots were used for all larval recovery data and the adult emergence data for the *S. faberi* experiment. Plastic 19-liter pots were used for adult emergence in the maize experiments. Drainage holes in both types of pots were covered with a fine (114 μm per opening) stainless steel mesh (TWP Inc., Berkley, CA) to prevent larval escapes (Clark and Hibbard 2004). A photoperiod of 14:10 (L:D) h was maintained with

1,000-W sodium bulbs (GE Lighting, Cleveland, OH). All plants were watered as needed and fertilized (Scotts Peters Professional 20–20–20, Scotts-Seirra Horticultural Products Corp., Marysville, OH) every 7 d. Temperature was recorded on an hourly basis (model H08-001-02, HOBO, Bourne, MA).

Larval Preparations. The nondiapausing strain of western corn rootworm larvae used for all experiments was obtained from a colony maintained in our laboratory that was subsidized as needed by eggs from the USDA–ARS Northern Grain Insects Research Laboratory (NGIRL) in Brookings, SD, and French Agricultural Research, Lamberton, MN. After recovery from oviposition dishes, western corn rootworm eggs were placed in 708-ml Gladware semidurable plastic containers (Clorox, Oakland, LA) covered by a thin layer of soil at 25°C until hatch began. Neonate larvae were infested within 24 h after they hatched. Second instars were infested on the same days as neonate larvae and were obtained by infesting 125 neonate larvae 6–8 d earlier (in one instance up to 11 d) on maize plants (≈15 g of untreated DKC 60-15 maize seed, Monsanto Company, St. Louis, MO) that were planted 4–5 d earlier than that in 708-ml containers described above. When needed, second-instar larvae were recovered by the use of Tullgren funnels. Second instars were infested within a few hours after they were first placed into the funnels. Both neonate and second-instar larvae were infested with a fine nylon brush, and the larvae were placed gently at the base of the maize or *S. faberi* plants. With each trial of each experiment, 100 reference larvae were randomly sampled from each colony used for that trial for both neonate and second-instar larvae. Reference larvae were stored in 95% ethanol before head capsule width and dry weight determination. If second instars of different ages were used for certain replications, these were sampled separately and the replicate that they were used for was recorded.

Larval Recovery. At 5, 10, and 15 d after infestation, the contents of designated pots (soil mixture, roots, and larvae) was placed in Tullgren funnels equipped with a 60-W light bulb (Great Value, Soft White, Wal-Mart Company) for the extraction of the larvae. Collection jars containing water were placed under each funnel. After 2 and 4 d, larvae were counted and transferred to scintillation vials containing 95% ethanol. The head capsule width (HCW) of each larva was measured using an ocular micrometer (10×/21, Wild Co., Heerbrugg, Switzerland) mounted on a microscope (M3Z, Wild Co.). Dry weights of the larvae were determined using an analytical scale (ER-182A, A and D Co., Tokyo, Japan) after placing the larvae in a desiccating oven (Thelco model 16, GCA/Precision Scientific Co., Chicago, IL) at 60°C for 48 h. The average weight of the larvae was calculated by taking the total weight of all larvae recovered per pot and dividing this number by the number of larvae. The average initial starting weight of the reference larvae described above was then subtracted from this average weight to obtain the change in weight for analysis.

Beetle Recovery. Beetle emergence pots were covered with mesh 1–2 d after the 15-d larval recovery to monitor adult emergence. Pots were checked for adults every 2 d until 2 wk after the last beetle was found. Control maize plants were allowed to grow for the whole experiment. The mesh was secured around the base of the maize plants with plastic zip ties. The *S. faberi* control plants were cut down to 4–5 cm above soil level and covered when the first adult was found on the maize control that was infested with the same larval stage. All adults collected were stored in 95% ethanol and kept until sex, head capsule width and dry weight could be recorded as described above.

Experiment 1. Glyphosate-Sprayed *S. faberi*

Trial 1. *S. faberi* seed (1 g per pot) was planted on 3, 8, and 13 February 2006 for treatment 6, treatment 5, and treatments 7, 1–4, respectively (Table 1). Maize controls (four seeds per pot) were planted on 8 February and thinned to two plants per pot 2 wk later. *S. faberi* plants were sprayed with a 2.4% solution of glyphosate solution (Touchdown Total, Syngenta, Basil, Switzerland) on 3 March (treatments 4 and 6), 8 March (treatments 3 and 5), and 13 March (treatment 2). *S. faberi* plants grew 20–30 cm in height depending on the treatment at the time of glyphosate spray. Designated pots were infested with 30 neonate larvae on 13 March (replications 1–3) or 14 March (replications 4–5). Designated pots were infested with eight second instar western corn rootworm larvae on 13 March. Unfortunately, the number of second-instar larvae ran low in this trial, and only seven larvae were used for the fourth replication and five larvae were used for the fifth replication. Larval recovery dates were 5, 10, or 15 d after infestation for both neonate and second-instar larvae. Greenhouse temperatures ranged from a minimum of 14°C to a maximum of 49°C with an average hourly reading of 26 ± 0.09°C (SE). Presumably soil temperatures were not as extreme on the high and low end.

Trial 2. *S. faberi* seed was planted on 26 and 31 May and 5 June 2006 for various treatments as described for trial 1. Maize controls were planted on 31 May and later thinned to two plants as described for trial 1. *S. faberi* plants were sprayed with glyphosate on 23 June (treatments 4 and 6), 28 June (treatments 3 and 5), and 3 July (treatment 2). *S. faberi* plants grew 30–40 cm in height depending on the treatment at the time of glyphosate spray. Designated pots were infested with 30 neonate or eight second instars on 3 July. Larval recovery dates were 8 July (5 d), 13 July (10 d), and 18 July (15 d). Greenhouse temperatures ranged from a minimum of 21°C to a maximum of 37°C with an average hourly reading of 27 ± 0.06°C (SE).

Experiment 2. Glyphosate-Sprayed Maize

Trial 1. Maize seeds were planted 17, 22, and 25 February 2006 for treatments 6, 5, and 1–4, respectively. Emerged plants were thinned to one plant per pot on 5 March. Maize plants were sprayed with

glyphosate on 31 March (treatments 4 and 6), 5 April (treatments 3 and 5), and 10 April (treatment 2). The maize growth stages varied from V6 to V8 depending on the treatment at the time of glyphosate spray. All pots designated for larval recovery were infested with 30 neonate larvae or 10 second-instar larvae on 10 April. The second-instar portion of the adult emergence pots also were infested on 10 April, but the pots designated for adult emergence from a neonate infestation were infested on 11 April. Larval recovery dates were 15 April (5 d), 20 April (10 d), and 25 April (15 d). Greenhouse temperatures ranged from a minimum of 11°C to a maximum of 42°C, with an average hourly reading of 25.4 ± 0.11°C (SE).

Trial 2. Maize was planted on 26 and 31 May and 5 June 2006 for treatments 6, 5, and 1–4, respectively. Emerged plants were thinned to one plant per pot on 12 June. Maize plants were sprayed with glyphosate on 7 July (treatments 4 and 6), 12 July (treatments 3 and 5), and 17 July (treatment 2). The maize growth stages varied from V7 to V11 depending on the treatment at the time of glyphosate spray. Designated pots were infested with 30 neonate larvae or 10-second instar larvae on 17 July. Larval recovery dates were 22 July (5 d), 27 July (10 d), and 1 August (15 d). Greenhouse temperatures ranged from a minimum of 18°C to a maximum of 42°C, with an average hourly reading of 28.1 ± 0.20°C (SE).

Experiment 3. Severed Maize

Trial 1. Maize seed was planted 6, 11, and 16 January 2006 for treatments 6, 5, and 1–4, respectively. Emerged plants were thinned to one plant/pot on 20 and 27 January. Maize plants were severed below the growing point on 17 February (treatments 4 and 6), 22 February (treatments 3 and 5), and 27 February (treatment 2). The maize growth stages varied from V5 to V7 depending on the treatment. Designated pots were infested with 30 neonate larvae or 10-second instar larvae. All second-instar larvae were infested on 27 February. Neonate larvae were infested on 26 February (replications 1–2) and 27 February (replications 3–5). Larval recovery dates were 3 and 4 February (5 d), 8 and 9 February (10 d), and 13 and 14 February (15 d). Greenhouse temperatures ranged from a minimum of 13°C to a maximum of 34°C with an average hourly reading of 23.8 ± 0.06°C (SE).

Trial 2. Maize was planted on Feb 17, 22, and 27, 2006 for the various treatments described above. Emerged plants were thinned to one plant/pot on 5 March. Maize plants were severed below the growing point on 31 March (treatments 4 and 6), 5 April (treatments 3 and 5), and 10 April (treatment 2). At the time they were severed, the maize growth stages varied from V6 to V8 depending on the treatment. Greenhouse temperatures ranged from a minimum of 11°C to a maximum of 42°C with an average hourly reading of 25.2°C ± 0.11 SE. All pots designated for larval recovery were infested with 30 neonate larvae or 10 s instar larvae on 10 April. For adult emergence pots, second instar larvae were infested on 10 April and neonate larvae were infested on 11 April. Larval recovery dates were 15 April (5 d), 20 April (10 d), and 25 April (15 d).

Statistical Analysis. The data were analyzed using PROC MIXED (SAS Institute 1990). A separate analysis was done for the number of larvae recovered, the average change in dry weight, and the number of adults emerged for both those pots infested with neonate larvae and those pots infested with second-instar larvae for each experiment. The number of larvae recovered and the average change in dry weight were analyzed as a randomized complete block split split-plot in space outlined in Steele et al. (1997). There were two trials for each experiment which were arranged as a 6 by 3 (treatment × sample date) factorial (experiment 1 was 7 by 3). For the analysis, the linear statistical model contained the main plot effect of trial, the subplot of treatment, the sub-plot effects of sample date, and all possible interactions. Trial × replications served as the denominator of F for testing the effects of trial, and replications × trial within treatment served as the denominator for testing the effects of treatment. The residual mean square served as the denominator of F for all other effects. Beyond the standard analysis of variance (ANOVA), we preplanned to compare sample dates within treatments and treatments within sample dates, so LSD values ($\alpha = 0.05$) were calculated taking the standard error of the difference and multiplying this by the t value at error degrees of freedom. Total adult emergence was analyzed as a split-plot in space. The linear statistical model contained the main plot effect of trial and the subplot effects of treatment.

Results

Experiment 1. Glyphosate-Sprayed *S. faberi*

Neonate Larval Recovery. Treatment ($F = 24.05$; df = 6, 48; $P < 0.0001$), treatment × sample date ($F = 2.24$; df = 12, 112; $P = 0.0141$) and treatment × trial × sample date ($F = 2.67$; df = 12, 112; $P = 0.0034$) significantly influenced the number of larvae recovered. The main effect of trial ($F = 2.54$; df = 1, 4; $P = 0.1863$) was not significant. The number of larvae recovered was significantly greater than zero for only the living maize and living *S. faberi* controls (Fig. 1A). Significantly more neonate larvae were recovered from the living maize control than the living *S. faberi* at the second and third, but not the first sample date (Fig. 1). Significantly more neonate larvae were recovered from the living maize and living *S. faberi* than any other treatment for all three sample dates (Fig. 1A). Treatments 2–6 did not differ significantly from zero on any sample date.

Neonate Weight Change. Treatment ($F = 83.20$; df = 6, 48; $P < 0.0001$), sample date ($F = 60.93$; df = 2, 112; $P < 0.0001$), and treatment × sample date ($F = 37.58$; df = 12, 112; $P < 0.0001$) significantly influenced the change in weight of the larvae recovered. The main effect of trial ($F = 0.45$; df = 1, 4; $P = 0.5410$) was not significant, but the interaction of treatment × trial

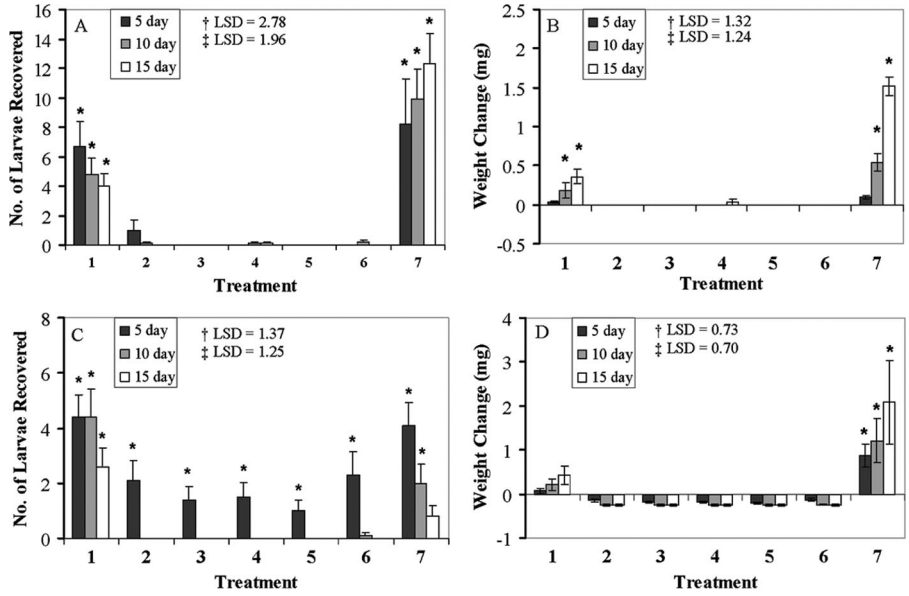

Fig. 1. Number of larvae recovered and change in dry weight from *S. faberi* and maize in experiment 1 (A, neonate larvae recovered; B, change in neonate dry weight; C, number of second-instar larvae recovered; and D, change in dry weight of second-instar larvae). See Table 1 for treatment list. An asterisk (*) indicates a significant number of larvae recovered or a significant weight change from zero. An dagger (†) indicates the least significant difference (LSD) ($\alpha = 0.05$) when comparing bars with the same recovery date but different treatments. A double dagger (‡) indicates the LSD ($\alpha = 0.05$) when comparing bars within the same treatments but different recovery dates.

was significant ($F = 2.35$; df = 6, 48; $P = 0.0452$). A significant positive weight change was recorded only for the living *S. faberi* and maize controls (treatments 1 and 7) and only on the second and third recovery dates (Fig. 1). The weight gain was significantly greater on living maize than living *S. faberi* on these dates and the weight gain on living *S. faberi* was significantly greater than from all sprayed treatments (treatments 2–6) on the second and third sample date.

Adult Emergence from Neonate Infestation. Treatment ($F = 35.47$; df = 6, 48; $P < 0.0001$), trial ($F = 12.68$; df = 1, 4; $P < 0.0236$), and treatment × trial ($F = 9.16$; df = 6, 48; $P < 0.0001$) all significantly affected the number of adults emerged. Adults were recovered only from the *S. faberi* and maize controls, averaging 0.4 ± 0.4 (SE) and 4.9 ± 1.1 (SE) beetles per pot, respectively (data not shown). The number of adults that emerged from living maize was significantly greater than the number that emerged from all other treatments. No other significant differences were found.

Second-Instar Recovery. Treatment ($F = 16.29$; df = 6, 48; $P < 0.0001$), sample date ($F = 35.79$; df = 2, 112; $P < 0.0001$), and trial × sample date ($F = 11.78$; df = 2, 112; $P < 0.0001$) had a significant influence on the number of larvae recovered. The effect of trial was also significant ($F = 9.57$; df = 1, 4; $P = 0.0148$), which was expected for this experiment because replications 4 and 5 in trial 1 were infested with fewer larvae. The number of larvae recovered was significantly greater than zero for all treatments on the first sample date, but only for the living controls for the other sample dates. The number larvae recovered from an infestation of second-instar larvae was significantly greater from treatment 1 than for all other treatments on the second and third sample dates. On sample date 1, there was no difference between treatment 1 and treatment 7. For treatment 7, some larvae likely began to pupate by the second sample date with most having done so by the third sample date (Fig. 1), accounting for the lower recovery from living maize on these dates.

Second-Instar Weight Change. Treatment ($F = 14.03$; df = 6, 48; $P < 0.0001$) significantly influenced the change in weight of the larvae recovered. No other factors in the model, including trial, were significant. Significantly more weight was gained on the maize control (treatment 7) than on any other treatment at each sample date (Fig. 1). Treatment 1 was the only other treatments with larvae that had a positive change in weight.

Adult Emergence from Second-Instar Infestation. Treatment ($F = 16.68$; df = 6, 48; $P < 0.0001$) significantly influenced the number of adults emerged, whereas trial and the interaction of trial × treatment did not significantly influence this. Adults were recovered only from the *S. faberi* and maize controls (data not shown). Treatments 1 and 7 infested with second-instar larvae averaged 0.3 ± 0.2 (SE) and 3.2 ± 0.7 (SE) beetles per pot for the *S. faberi* and maize controls, respectively. Significantly more adults were recovered from maize than *S. faberi* for both infestations with neonate ($F = 35.47$; df = 6, 48; $P < 0.0001$) and second-instar larvae ($F = 16.68$; df = 6, 48; $P < 0.0001$). The number of adults that emerged from

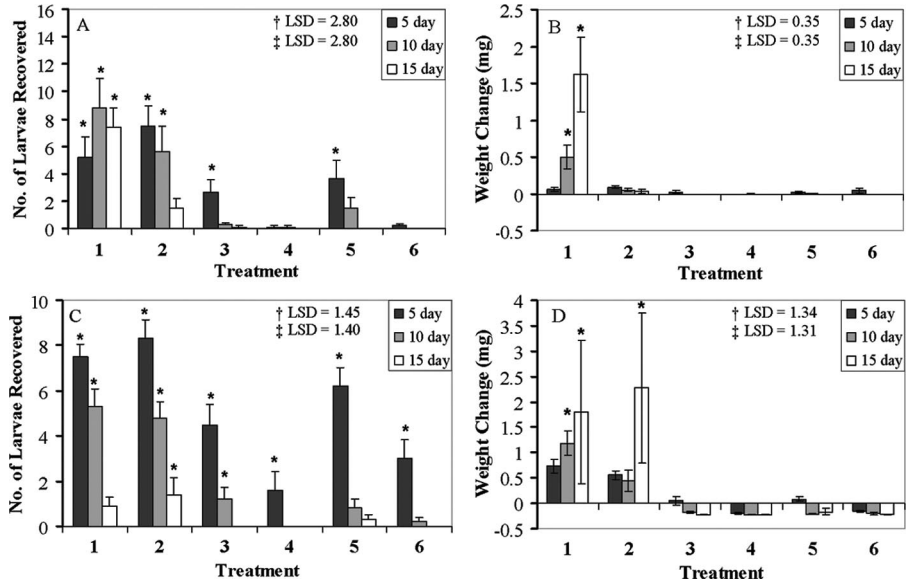

Fig. 2. Number of larvae recovered and change in dry weight from maize in experiment 2 (A, neonate larvae recovered; B, change in neonate dry weight; C, number of second-instar larvae recovered; and D, change in dry weight of second instar larvae). See Table 1 for treatment list. An asterisk (*) indicates a significant number of larvae recovered or a significant weight change from zero. A dagger (†) indicates the LSD ($\alpha = 0.05$) when comparing bars with the same recovery date but different treatments. A double dagger (‡) indicates the LSD ($\alpha = 0.05$) when comparing bars within the same treatments but different recovery dates.

living maize was significantly greater than the number that emerged from all other treatments. No other significant differences were found.

Experiment 2. Glyphosate-Sprayed Maize

Neonate Larval Recovery. Treatment ($F = 25.65$; df = 5, 40; $P < 0.0001$), sample date ($F = 4.71$; df = 2, 96; $P = 0.0112$), and treatment × sample date ($F = 2.72$; df = 10, 96; $P = 0.0055$) significantly affected the number of larvae recovered. Treatments 1, 2, 3, and 5 all recovered significantly more larvae than zero on the first recovery date (Fig. 2A). However, only from treatments 1 and 2 were significantly more larvae than zero recovered on the second sample date and only from treatment 1 for the third sample date (Fig. 2A). Although the difference was not significant, more larvae were recovered from the treatment planted 5 d early (treatment 5) than its analogous treatment (treatment 3); both treatments were sprayed with glyphosate 5 d before infestation.

Neonate Weight Change. Treatment ($F = 17.02$; df = 5, 40; $P < 0.0001$), sample date ($F = 5.88$; df = 2, 96; $P = 0.0039$) and treatment × sample date ($F = 7.40$; df = 10, 96; $P < 0.0001$) significantly affected the weight change in the larvae recovered. The live control (treatment 1) was the only treatment with a significant weight gain (Fig. 2). Although a significant number of larvae from the neonate infestation were recovered from treatments 2, 3, and 5, weight gain was not significantly different from zero for any sample date.

Adult Emergence from Neonate Infestation. The living maize control (treatment 1) was the only treatment on which adults were recovered (data not shown). An average of 4.0 ± 1.48 (SE) beetles per pot were recovered, but all of these were from trial 1, where an average of 8.8 ± 1.38 (SE) per pot were recovered. Treatment ($F = 33.68$; df = 5, 40; $P < 0.0001$), trial ($F = 33.68$; df = 1, 4; $P < 0.0044$) and trial × treatment ($F = 33.68$; df = 5, 40; $P < 0.0001$) significantly influenced the number of adults emerged.

Second-Instar Larval Recovery. Treatment ($F = 32.00$; df = 5, 40; $P < 0.0001$), treatment × trial ($F = 3.07$; df = 5, 40; $P = 0.0194$), sample date ($F = 142.35$; df = 2, 96; $P < 0.0001$), treatment × sample date ($F = 6.51$; df = 10, 96; $P < 0.0001$) and treatment × trial × sample date ($F = 2.20$; df = 10, 96; $P < 0.0237$) significantly affected the number of larvae recovered. When comparing treatments with an LSD, the number of second-instar larvae recovered from the living maize control (treatment 1) was not significantly different from the number of larvae recovered from treatment 2 on any sample date nor from the number of larvae recovered from treatment 5 on the first sample date (Fig. 2). Significantly more larvae were recovered from treatment 5 than treatment 3 on the first sample date.

Second-Instar Weight Change. The main effect of treatment ($F = 5.64$; df = 5, 40; $P = 0.0005$) significantly affected the change in larval weight, but the main effects of trial, sample date did not, nor did any of their interactions. There was no significant differ-

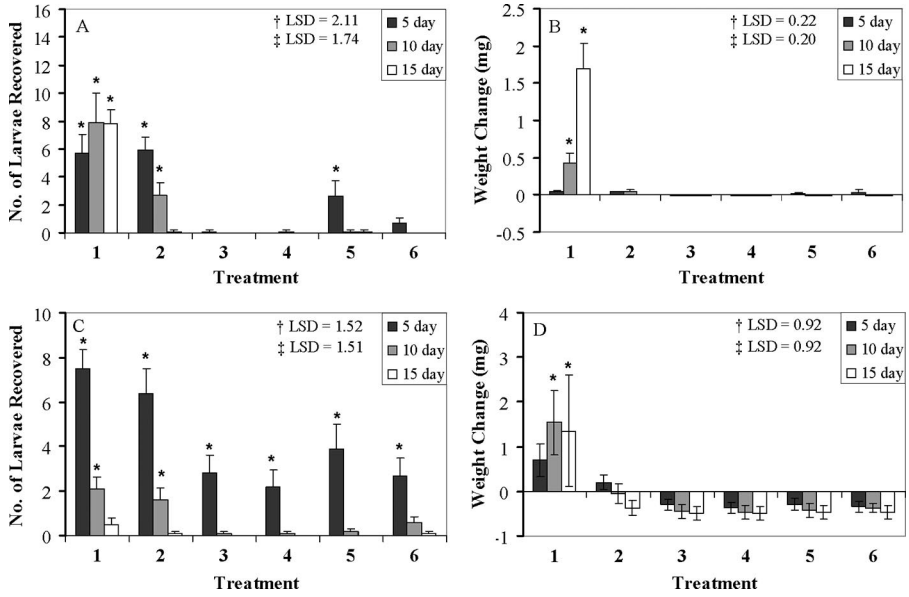

Fig. 3. Number of larvae recovered and change in dry weight from maize in experiment 3 (A, neonate larvae recovered; B, change in neonate dry weight; C, number of second-instar larvae recovered; and D, change in dry weight of second instar larvae). See Table 1 for treatment list. An asterisk (*) indicates a significant number of larvae recovered or a significant weight change from zero. A dagger (†) indicates the LSD ($\alpha = 0.05$) when comparing bars with the same recovery date but different treatments. A double dagger (‡) indicates the LSD ($\alpha = 0.05$) when comparing bars within the same treatments but different recovery dates.

ence in weight change between treatments 1 and 2 on any of the three sample dates (Fig. 2). Treatments 3 and 5 had a slightly positive weight gain for the first sample date. A negative change in weight was recorded for all larvae recovered from treatments 4 and 6, which were sprayed with glyphosate 10 d before infestation.

Adult Emergence from Second-Instar Infestation. The maize controls were the only treatments on which adults were recovered (data not shown). An average of 4.0 ± 1.22 (SE) beetles per plot emerged. Treatment ($F = 29.09$; df = 5, 40; $P < 0.0001$), trial ($F = 16.36$; df = 1, 4; $P < 0.0155$) and trial × treatment ($F = 16.36$; df = 5, 40; $P < 0.0001$) significantly influenced the number of adults emerged.

Experiment 3. Severed Maize

Neonate Recovery. Treatment ($F = 25.97$; df = 5, 40; $P < 0.0001$), sample date ($F = 5.44$; df = 2, 96; $P = 0.0058$), and treatment × sample date ($F = 5.35$; df = 10, 96; $P = 0.0001$) all significantly affected the number of neonate larvae recovered, but other factors in the model did not. The number of larvae recovered was significantly greater than zero for all sample dates from the living maize control, certain sample dates from treatments 2 and 5. On the first sample date, more larvae were recovered from treatments 5 and 6 than treatments 3 and 4 respectively (Fig. 3), implying that the size of the root mass of the plant had an effect on the number of larvae recovered. The comparison of treatments 5 and 3 on the first sample date was significant (Fig. 3).

Neonate Weight Change. Treatment ($F = 32.82$; df = 5, 40; $P < 0.0001$), treatment × trial ($F = 3.66$; df = 5, 40; $P = 0.0080$), sample date ($F = 21.11$; df = 2, 96; $P < 0.0001$), treatment × sample date ($F = 24.54$; df = 10, 96; $P = < 0.0001$) and treatment × trial × sample date ($F = 2.59$; df = 10, 96; $P = 0.0080$) all had a significant influence on the change in weight of the larvae recovered. Treatments 2, 5, and 6 had a slight positive change in weight on one or sample dates, but only treatment 1 had a change in weight that was significantly positive (Fig. 3). The weight of larvae recovered from treatment 1 was significantly different than the weight of larvae from any other treatment at sample date 2 and 3.

Adult Emergence from Neonate Infestation. The living maize control (treatment 1) was the only treatment on which adults were recovered (data not shown). An average of 7.80 ± 1.5 (SE) beetles per pot were recovered. Treatment ($F = 24.10$; df = 5, 40; $P < 0.0001$) significantly influenced the number of adults emerged, but trial and the interaction of trial and treatment did not.

Second-Instar Recovery. Treatment ($F = 11.51$; df = 5, 40; $P < 0.0001$), sample date ($F = 103.17$; df = 2, 96; $P < 0.0001$), trial × sample date ($F = 5.87$, df = 2, 96; $P = 0.0039$), and treatment × sample date ($F = 3.86$; df = 10, 96; $P = 0.0002$) significantly influenced the number of larvae recovered. Treatments 1 and 2 were the only two treatments to have a significant number of larvae recovered on the second sample date (10 d) (Fig. 3). The live maize control (treatment 1) had decreasing larval recovery numbers on the second and third sample date (10 and 15 d), probably because of

some larvae entering pupation. Treatments 1 and 2 had no significant difference between them on any sample date.

Second-Instar Weight Change. The main effect of treatment ($F = 11.79$, df = 5, 40; $P < 0.0001$) had a significant influence on the number of larvae recovered. No other main effect or interaction significantly affected the change in weight of second-instar larvae. Treatments 1 and 2 were not significantly different on the first sample date and were the only two treatments to have a positive change in weight change on this sample date (Fig. 3).

Adult Emergence from Second-Instar Infestation. Adults were recovered from all treatments, but only treatments 1 and 2 had adults recovered from both trials (data not shown). Treatment ($F = 24.10$; df = 5, 40; $P < 0.0001$) significantly influenced the number of adults emerged, but trial and the interaction of trial and treatment did not. An average of 6.4 ± 0.6 (SE) beetles per pot were recovered from the control (treatment 1), which was significantly greater than all the other treatments. An average of 1.9 ± 0.7 (SE) beetles per pot were recovered from treatment 2, which was significantly greater than the remaining treatments. Treatments 3, 4, 5, and 6 averaged less than one beetle per pot and had no significant differences between them.

Discussion

In the current study, the ability of living *S. faberi* to support neonate and second-instar larvae for a positive weight change on all three sample dates indicates its suitability as an alternate host, which has been documented previously (Clark and Hibbard 2004, Wilson and Hibbard 2004). However, when *S. faberi* was sprayed with glyphosate, neonate western corn rootworm survival and weight gain was minimal (Fig. 1). Second-instar western corn rootworm larvae had a significant number of larvae recovered on the first sample date (5 d) of all treatments, but there was no positive weight gain for any *S. faberi* treatments sprayed with glyphosate on any sample date (Fig. 1). These results indicate that within the first 5 d after *S. faberi* was sprayed with glyphosate, it no longer supported western corn rootworm growth and development for either neonate or second-instar larvae. Recovery of second instars on the first sample date can be attributed to the ability of the larger larvae to withstand a longer period of starvation.

Why is it that a plant that is just beginning to show symptoms of being sprayed with glyphosate is no longer suitable as a host for western corn rootworm development? At this point, it is speculation, but glyphosate does inhibit amino acid biosynthesis and the translocation of sucrose and proteins (Nadler-Hassar et al. 2004). Because sucrose is an important component of a feeding stimulant blend for western corn rootworm larvae that also includes long-chain free fatty acids (Bernklau and Bjostad 2008), it could be that western corn rootworm larvae do not eat glyphosate-sprayed plants. Amino acids and/or proteins are obviously important components of insect diets (Singh and Moore 1985). It is also possible that essential amino acids or protein components are no longer present in roots recently sprayed with glyphosate. Peterson et al. (2007) reported that levels of glyphosate that are much lower than the level required to produce visible symptoms produce dramatic metabolic effects on rapeseed seedlings. Perhaps similar changes occur in maize that affect western corn rootworm development.

All previous studies evaluating maize and alternate hosts together have concluded that maize is a superior host for western corn rootworm larvae than any alternate host evaluated to date (Moeser and Hibbard 2005). Although experiments 1–3 cannot be directly compared statistically, dying maize (both sprayed with glyphosate and severed below the growing point) seemed to support larval survival and weight gain better than dying *S. faberi* that had been sprayed with glyphosate (Figs. 1–3). However, as indicated by the overall lack of weight gain, dying maize and dying *S. faberi* are vastly inferior hosts when compared with living tissue (Figs. 1–3).

The amount of time that dying roots of maize and *S. faberi* supported growth and development of western corn rootworm larvae in our study may provide insight into the interpretation of data obtained by Moeser and Vidal (2004a,b). Moeser and Vidal (2004a,b) evaluated severed maize and alternate host roots in their experiments for 6 d. In our study, maize roots infested 5 d after the plant was severed below the growing point did not support significant positive weight gain of neonate or second-instar western corn rootworm larvae (Fig. 3). The weight gain of both neonate and second-instar larvae was significantly less on plants that were severed below the growing point on the day of infestation than living maize plants after being allowed to feed for 10 d, but this difference was not significant when allowed to feed for only 5 d (Fig. 3). Neither neonate nor second-instar larvae had any significant positive weight gain when infested on *S. faberi* the day that it was sprayed with glyphosate, although differences in weight gain from living *S. faberi* were not statistically significant (Fig. 1). We did not evaluate all the maize varieties nor alternate host species evaluated by Moeser and Vidal (2004a,b) in the current experiment, but our data imply that differences between maize varieties and alternate hosts found by Moeser and Vidal (2004a,b) could have been confounded by differences in the rate at which root tissues of the maize varieties (Moeser and Vidal 2004b) and alternate hosts (Moeser and Vidal 2004a) die after removal from the plant.

In experiment 3, because maize was severed near ground level (below the growing point), symptoms of aboveground plant death were quite fast. Maize sprayed with glyphosate did not show signs of aboveground plant death until several days after they were sprayed. The timing of plant death could easily account for any differences between experiments 2 and 3. The reason for the differences between the glyphosate-sprayed maize and glyphosate-sprayed *S.*

faberi in how long and well they support western corn rootworm growth and development is unknown. Some possible factors may include the difference in the diameter of the roots, the suitability of the host plant in general, or the time needed for the glyphosate to kill the particular plant species. Time to initial herbicide symptoms in *S. faberi* was shorter than for maize.

Adult emergence in all studies was found primarily in living control plants. Only one of the three experiments had adults emerge from any treatment other than living plants. In experiment 3, where maize was severed below the growing point, adults were recovered from all treatments when infested with larger larvae. This is understandable when we examined the head capsule width of the larvae used to infest the pots where adults emerged. The head capsule width of reference larvae kept for initial weight indicated that they were actually third instars at the time of infestation for three of the 10 replication. Except for living control plants, adults were only recovered from these three replications of trial 1 that were infested with larger larvae. A few adults were recovered from treatment 2 when infested with second-instar larvae in trial 2 of experiment 3. These larvae were initially all second instar larvae as indicated by head capsule width of the reference larvae. The lack of any adults being recovered from any treatment that was sprayed with glyphosate in experiment 2 is somewhat surprising because maize sprayed with glyphosate supported larval survival and positive weight change longer than the severed maize plants.

In field tests where maize plants were severed at the base while *D. v. virgifera* were in the pupal stage, the number of adults emerging from severed and living plants was not significantly different; however, the emergence rate was accelerated (Fisher 1984). Presumably, the accelerated pace of adult emergence resulted from larvae that had nearly completed development pupating early when food quality became poor. Our results from the severed maize experiment would tend to support these findings when the majority of the larvae are in the third-instar stage. In the few cases where we evaluated early third-instar larvae (replications 1–3, trial 1 of experiment 3), adults were produced. However, when larvae were neonate or second-instar larvae, adults were generally not produced.

Oyediran et al. (2005) documented in greenhouse experiments that significantly more western corn rootworm beetles were produced from a combination of rootworm-resistant transgenic maize plus grassy weeds than the transgenic maize alone or the weeds alone. Oyediran et al. (2007) evaluated combinations of grassy weeds and transgenic maize in a field setting, but damage to and adult emergence from *Bt* maize did not vary with grassy weed species or herbicide application time. The current experiments evaluated larval growth and development in no-choice tests, whereas Oyediran et al. (2005, 2007) presented various combinations of treatments at the same time. Because the number of larvae recovered from *S. faberi* in the current experiment was not significantly greater than zero for neonate infestations, even after only 5 d (Fig. 1), the current data imply that western corn rootworm larvae must seek a different host relatively quickly when the host currently being fed upon is sprayed with herbicide.

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